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Potential for wild species in cool season food legume breeding

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Abstract

Wild species which are crossable to cultivated pea, lentil, and chickpea have been collected and are maintained in major germplasm collections throughout the world. Wild species of *Vicia* crossable to the cultivated faba bean have not been found. The primary, secondary, and tertiary gene pools of the cool season food legumes represent potential genetic diversity that may eventually be exploited in cultivated types to overcome biotic and abiotic stresses. Technical difficulties in obtaining hybrids beyond those within the primary gene pool is a major obstacle. Reproductive isolation, embryo breakdown, hybrid sterility, and limited genetic recombination are major barriers to greater use of wild germplasm. Conventional crossing has been successful in producing interspecific hybrids in *Lens*, *Cicer* and *Pisum* and those hybrids are being evaluated for desired recombinants. *In vitro* culture of hybrid embryos has been successful in overcoming barriers to wider crosses in *Lens*. The successful transfer of genes from wide sources to cultivated types can be assisted by repeated backcrossing and selection designed to leave behind undesired traits while transferring genes of interest. Molecular marker assisted selection may become a valuable tool in the future use of wild species. In general, too little is known about the possible genetic variation available in wild species that could be valuable in developing resistance to biotic and abiotic stresses. Current efforts on the use of wide hybridization in the cool season food legumes are reviewed and discussed.

Introduction

The wild species progenitors of the cool season food legumes have been found and collected in the presumed centers of origin in the Near East and in the nearby areas of southern Europe and central Asia (Ladizinsky *et al.*, 1988; Muehlbauer *et al.*, 1989; Muehlbauer *et al.*, 1990; Van der Maesen and Pundir, 1984). Wild relatives closely related to cultivated pea, lentil, and chickpea have been collected. These accessions have the potential to provide the needed genetic variation for the improvement of these crops for resistance to many of the biotic and abiotic stresses that have been particularly troublesome. In addition, the wild relatives may provide a means of expanding the range of these crops to previously unsuitable areas. Genes for tolerance to cold temperatures, tolerance to heat and drought, and resistance to disease and insect pests, may enable cool season food legumes to be grown successfully in previously unsuitable environments.

There is a belief that the collections of the cultivated materials have not been fully utilized in breeding programs (Hawtin *et al.*, 1988), and that breeders should concentrate on cultivated germplasm before looking to the wild relatives. This is especially true where needed genetic variation is known to be present in cultivated material. Nevertheless, the wild species accessions that are available have been subjected to crossability studies, cytological examinations, and limited evaluations for needed genetic variation. The findings have provided an indication of what may be possible by conventional methods and what may require complex procedures.

Important considerations for the exploitation of the wild species to alleviate biotic and abiotic stresses in the food legumes include: the placement of the rele-

vant species into gene pools relative to the cultigen, barriers to interspecific hybridization and how those barriers might be overcome, evaluation of wild species for traits of importance, and techniques of gene transfer if conventional crossing is not possible.

Gene pools of the cool season food legumes

Even though species closely related to the cultivated cool season food legumes have been collected and are being maintained in gene banks for use by breeders, crossability barriers have limited their usefulness. A systematic means of categorizing wild species as to their usefulness for improving the cultigen has been formulated by Harlan & De Wet (1971). According to the concept, the *primary* gene pool of a cultivated species is equivalent to a biological species. Species within the primary gene pool are readily intercrossed and produce progenies that are fully fertile or nearly so. Consequently, gene flow between species of the primary gene pool can be accomplished by conventional breeding methods. Any partial fertility that appears is easily overcome by selection among the progenies.

The *secondary* gene pool contains species which are somewhat distant from the cultigen. Hybridization, to obtain gene flow, is more difficult and the progenies have substantial degrees of sterility, usually because of chromosomal rearrangements.

The *tertiary* gene pool contains those species that are related to the cultigen but where hybridization with the cultigen has not been possible or where hybrids have been completely sterile. Species in the primary, secondary and tertiary gene pools of the cool season food legumes are shown in Table 1.

Lentil

The wild *Lens* species and the cultigen, *L. culinaris* are all diploid (2n = 14 chromosomes) and are predominantly self pollinators. In addition, all *Lens* species have a similar karyotype consisting of one metacentric chromosome with a satellite, three metacentric chromosomes, and three acrocentric chromosomes (Ladizinsky & Sakar, 1982).

All the wild *Lens* species are considered to be crossable to the cultigen (Ladizinsky *et al.*, 1988), but there is difficulty in obtaining certain hybrids (Table 1). Hybridizations are readily obtained between *L. culinaris* ssp. *culinaris*, the cultigen, and ssp. *orientalis* and ssp. *odemensis*; but, embryo rescue is needed to obtain hybrids of the cultigen with *L. nigricans* ssp. *ervoides* or *L. nigricans* ssp. *nigricans* (Cohen *et al.*, 1984; Ladizinsky *et al.*, 1985). Therefore, the subspecies of *L. nigricans* are considered to be in a secondary gene pool. Progenies have partial fertility but fully fertile segregants can be selected. Accessions of wild *Lens* are available from the US and ICARDA germplasm collections.

Chickpea

The annual wild *Cicer* species, including the cultigen, *C. arietinum*, are all diploid (2n = 16 chromosomes) with similar karyotypes, and are predominantly self pollinators. *Cicer reticulatum* and *C. echinospermum* are included in the primary gene pool of cultivated chickpea (Table 1). Hybridizations of *C. arietinum* with *C. reticulatum* are readily obtained and the progenies are fully fertile. *Cicer echinospermum* is crossable to *C. arietinum* but the hybrids are completely self-sterile due to the presence of a reciprocal translocation (Ladizinsky *et al.*, 1988). Seeds can be obtained by backcrossing to *C. arietinum*. It has been noted that certain accessions of *C. arietinum* produce partially fertile hybrids when crossed to *C. echinospermum* (Muehlbauer, personal observation). Very little work has been done with the perennial *Cicer* species, possibly because of difficulties in producing seeds and maintaining the accessions. *Cicer anatolicum* has shown good resistance to Ascochyta blight in screening trials (Muehlbauer and Kaiser, unpublished) and could provide an additional source of variation for controlling that disease.

Attempts to cross *C. arietinum* with the remaining annual *Cicer* species (*C. bijugum*, *C. pinnatifidum*, *C. judaicum* and *C. chorassanicum*) have been unsuccessful, and therefore these species are considered to be in the tertiary gene pool.

Pea

All *Pisum* species are diploid (2n = 14 chromosomes), share a similar karyotype, and are predominantly self-pollinators. All the subspecies of *Pisum sativum* are crossable to the cultigen, *P. sativum* ssp. *sativum*, all of which comprise the primary gene pool (Table 1). There is little difficulty in the use of *P. fulvum* in crosses with subspecies of *P. sativum* when *P. fulvum* is used as the pollen parent.

Table 1. Species in the primary, secondary and tertiary gene pools of lentil, chickpea, pea and faba bean

Crop	Gene pool		
	Primary	Secondary	Tertiary
Lentil	*Lens culinaris* ssp. *culinaris* *Lens culinaris* ssp. *orientalis* *Lens culinaris* ssp. *odemensis*	*Lens nigricans* ssp. *nigricans* *Lens nigricans* ssp. *ervoides*	Not known
Chickpea	*Cicer arietinum* *Cicer reticulatum* *Cicer echinospermum*		*C. bijugum* *C. pinnatifidum* *C. judaicum* *C. chorassanicum* *C. montbretii*
Pea	*Pisum sativum* ssp. *sativum* *Pisum sativum* ssp. *elatius* (including ssp. *humile*	*Pisum fulvum*	Not known
Faba bean	*Vicia faba*	None known	*V. narbonensis* *V. hyaeniscyamus* *V. galilaea* *V. johannis* *V. bithynica*

Faba bean

Vicia faba has 2n = 12 chromosomes and generally exhibits a high percentage of outcrossing. The wild progenitor of faba bean has not been found and there are no wild *Vicia* species which are crossable to the cultigen. Distantly related *Vicia* species might be placed in the tertiary gene pool (Table 1).

Evaluations of wild species

Lentil

Collections of wild lentil are available and there has been increased interest in evaluation for resistance to important diseases. Bayaa *et al.* (1991) systematically screened the ICARDA wild lentil collection for resistance to Fusarium wilt caused by *Fusarium oxysporum* f. sp. *lentis* and for resistance to Ascochyta blight caused by *Ascochyta fabae* f. sp. *lentis* (= *A. lentis*). Considerable genetic variation was found for these two diseases even though the majority of the accessions were susceptible. Resistance to Fusarium wilt was identified in 3 of 109 accessions of *L. culinaris* ssp. *orientalis*, 3 of 30 accessions of *L. nigricans* ssp. *nigricans*, and 2 of 63 accessions of *L. nigricans* ssp. *ervoides*. Resistance to Ascochyta blight was identified in 24 of 86 accessions of *L. culinaris* ssp. *orientalis*, 12 of 35 accessions of *L. culinaris* ssp. *odemensis*, 3 of 35 accessions of *L. nigricans* ssp. *nigricans* and 39 of 89 accessions of *L. nigricans* ssp. *ervoides*. One accession of *L. nigricans* ssp. *ervoides*, ILWL 138, had combined resistance to both diseases; however, the use of that accession for breeding combined resistance to Fusarium wilt and Ascochyta blight may be difficult because of the need to use embryo culture to obtain hybrids with the cultigen. It would be straight forward to utilize resistant accessions of ssp. *orientalis* resistant to one of the diseases and other accessions with resistance to the other disease even though additional crosses would be required to obtain combined resistance.

Screening of wild *Lens* against *Orobanche* has not revealed accessions with resistance (ICARDA, 1991).

Chickpea

Collections of the wild species of *Cicer* are maintained in genebanks and some have been evaluated for impor-

tant disease and insect pests. In a limited evaluation of seven *Cicer* spp., one accession each of *C. pinnatifidum*, *C. montbretii* and *C. judaicum* were highly resistant to Ascochyta blight (Singh *et al.*, 1981), while accessions of *C. yamashitae*, *C. bijugum*, *C. cuneatum* and *C. reticulatum* were tolerant to highly susceptible.

Resistance to cyst nematode, *Heterodera ciceri* was identified in 21 accessions of *C. bijugum*, five accessions of *C. pinnatifidum* and, most importantly, in one accession of *C. reticulatum* (Singh *et al.*, 1989; Singh & Reddy, 1991). The finding of resistance in *C. reticulatum* is of particular interest because of the ease of hybridization with the cultigen.

In an evaluation of annual wild *Cicer* species for cold tolerance, most accessions of *C. bijugum*, *C. echinospermum* and *C. reticulatum* were tolerant and were better than the cultigen (Singh *et al.*, 1991). All accessions of *C. chorassanicum*, *C. cuneatum*, *C. yamashitae*, and all but one accession of *C. judaicum* were susceptible. Accessions of *C. pinnatifidum* had both susceptible and tolerant reactions. Of these accessions, those of *C. reticulatum* and *C. echinospermum* are likely to be the most valuable for breeding, at least in the short term, because they are readily crossable to the cultigen. Use of genes present in the other wild *Cicer* species will need to await the development of techniques for obtaining hybrids or more novel means of gene transfer.

Other resistances found in evaluations of wild *Cicer* species include: resistance to Fusarium wilt in *C. judaicum* (Nene & Haware, 1980), and resistance to Botrytis gray mold (Singh, G. *et al.*, 1982).

Pea

All known accessions of wild *Pisum* are readily crossable to the cultigen; however, in using *P. fulvum* it is important to use the wild species as the pollen parent. Recently, there has been increased interest in *P. fulvum* as a possible source of resistance to bruchids (and see Clement *et al.*, 1994, this issue).

Faba bean

There are no reported cases of interspecific hybridization between cultivated faba bean and any of the wild *Vicia* species. Apparently, pollen tubes are able to reach and fertilize the ovules, but post-zygotic barriers prevent hybrid embryo development (Pickersgill *et al.*, 1985). Unfortunately, the wild progenitor of faba bean is yet to be discovered. *Vicia* species in the tertiary gene pool (Table 1) might have the needed variation for such traits as resistance to rust, chocolate spot and possibly *Orobanche* spp. (Ladizinsky *et al.*, 1988). Success in obtaining interspecific crosses between *V. faba* and the other *Vicia* species would provide much needed genetic variation for improving faba bean.

Introgression using molecular markers

Introgression of single genes from wild species into crop species involves the transfer of segments of chromosomes of various sizes into the recipient genome. These segments occur not only proximate to the target gene (linkage drag), but also in other locations throughout the genome (Stam & Zeven, 1981). Donor genome DNA other than the gene of interest may be deleterious and warrant elimination. The recent development of molecular marker systems and linkage maps are important tools for transferring genes of interest while preventing the transfer of genes which may be undesirable but closely linked.

Molecular marker systems, by their very nature, offer direct examination of the introgressed genotype at any given locus. Loci under examination with these systems may or may not have an overt phenotypic effect, but may offer a subtle reversion of plant type toward the wild phenotype due to the infusion of wild species DNA. Because molecular marker systems directly examine the recipient genotype, they have the capacity to detect the presence of donor genome DNA that may have only subtle but additive deleterious effects. Furthermore, markers can analyze the size and location of residual donor genome carried along through gene introgression and provide the breeder additional information for monitoring the elimination of unwanted donor DNA. The most elegant illustration of the use of molecular markers for this purpose can be found in the review article by Tanksley *et al.* (1989), wherein the authors performed computer simulations to show that while traditional backcross breeding may reach 99% recurrent genome in 6.5 generations, marker assisted backcrossing can approach 100% recurrent genome in three generations. They also illustrate that while it would take 100 backcrosses to reduce linkage drag to 1 cM by conventional backcrossing, it could be done in two backcrosses by marker assisted backcrossing in the context of a highly saturated linkage map.

Molecular markers are most useful in the context of a well developed genetic linkage map. Such maps

have been developed for a number of cool season food legume genera, including *Pisum* (Weeden & Wolko, 1990; Ellis *et al.*, 1992), *Lens* (Havey & Muehlbauer, 1989; Weeden *et al.*, 1992; Simon *et al.*, 1993), and *Cicer* (Simon & Muehlbauer, 1991). Maps for each of these genera currently consist of approximately 100 loci each (more in pea) and several research groups have indicated that they intend to greatly expand the chickpea map (Muehlbauer and Simon, personal communications).

The maps that have been developed for lentil and chickpea are particularly useful for gene introgression from wild relatives, since these maps have been developed from interspecific crosses of the cultivated species with its putative progenitor species. Havey & Muehlbauer (1989) developed the first RFLP map of lentil by analyzing crosses of *L. culinaris* ssp. *culinaris* with *L. culinaris* ssp. *orientalis*. Weeden *et al.* (1992) expanded upon that by analyzing crosses of *L. culinaris* by *L. ervoides* provided by G. Ladizinsky. Similarly, Simon & Muehlbauer (1991) have developed a map of *Cicer* by analyzing crosses of *C. arietinum* by *C. reticulatum*. In so doing, these authors have already identified numerous molecular polymorphisms between the respective species. In the case of *Cicer*, the resulting map will be especially useful because of the extremely high uniformity of the molecular morphology of the cultivated species, *C. arietinum*.

Molecular marker systems used in the cool season food legumes include isozymes (Zamir & Ladizinsky, 1984; Weeden & Marx, 1987; Ahmad *et al.*, 1992), restriction fragment length polymorphisms (RFLPs) (Ellis *et al.*, 1992; Havey & Muehlbauer, 1989; Simon & Muehlbauer, 1991) and most recently, randomly amplified polymorphic DNAs (RAPDs) (Simon & Muehlbauer, 1992; Weeden *et al.*, 1992; Simon *et al.* 1993).

The relative merits of isozymes, RFLPs, and RAPDs for assisting in gene transfer can be debated, but isozymes and RAPDs seem to be the most useful and practical. While isozymes are comparatively simple, the number of polymorphisms available for use is quite limited and linkages sufficiently close are often not found. The RAPD system offers the advantage of simplicity and a sufficiently large number of polymorphisms that close linkages to genes of interest can most often be found. Repeatability of the RAPD system is sometimes called into question and it appears that RAPD loci are often found in clusters within the genome. The RFLP system has the advantage of repeatability, but the technical difficulties of applying the method in practical breeding and the usual need for radioactive ^{32}P has meant that breeders have generally not adopted the method.

Concluding remarks

Wild species become of interest to plant breeders when desired gene(s) are not available in the cultigen. This seems to be the case with the cool season food legumes, particularly with regard to resistance to Ascochyta blight of chickpea and lentil, rust of lentil and faba bean, nematodes of chickpea, tolerance to heat, cold and drought of chickpea, resistance to *Orobanche* ssp., and resistance to insects. Fortunately, the wild species relatives of the cool season food legumes have been collected and are available for evaluation and breeding purposes. Additional targeted collection of wild species should be undertaken to expand on this genetic resource, but nevertheless it is becoming apparent that useful variation is already available.

Only a limited number of species are crossable to the cultigen; and, where crossing is successful, the resulting progenies must undergo stringent selection and backcrossing to recover useful material with the gene of interest from the wild species source. In most cases, the gene of interest in the wild species is linked to undesireable and deleterious genes and there is difficulty in achieving desired recombinations. These technical difficulties can be formidable and result in limited use of wild species germplasm.

Molecular marker facilitated introgression is an emerging breeding tool which can be used effectively in selection of progenies from interspecific hybridization. Specifically, molecular markers known to be close to the gene(s) of interest can be used to break undesireable linkages and eliminate unwanted or deleterious genes. It is fortunate that the wild species relatives of the cool season food legumes are available for breeding purposes. Techniques of gene transfer using molecular markers should allow breeders to use this genetic resource with the expectation of obtaining useful recombinants within a reasonable period of time.

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